FURTHER EXPERIMENTS ON THE EFFECT OF HEAT ON THE EGGS OF CUMINGIA.

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I. *Introduction.*—If the eggs of the mollusc *Cumingia tellinoides* are heated during the prophase of the first maturation division, the formation of the polar body is prevented. The nuclear division continues, giving rise to two daughter nuclei, both of which are retained in the egg. In the unfertilized egg these nuclei fuse, so that the egg is in fact fertilized by the polar nucleus. The course of the development which follows this self-fertilization has already been described (Morris, '17). Since the publication of that study, experiments similar to those producing artificial parthenogenesis have been made on fertilized eggs. It was found that if eggs are heated immediately after insemination they do not form polar bodies, but proceed to the first cleavage at the end of the warming. As these eggs should contain a triploid instead of a diploid number of chromosomes, it was thought that a study of them and a comparison with dispermic eggs might yield interesting results. Theoretically, of course, the polar nucleus is equivalent to a sperm nucleus, and either of these is equivalent to the egg nucleus. The suppression of maturation gives a chance for an interesting comparison between the sperm and polar nuclei. Besides the experiments in which maturation was suppressed, a control series of experiments was made to distinguish the effects of heating from those of the retention of the polar nucleus. In this series, the fertilized eggs were heated after they had completed the formation of polar bodies. Any abnormalities found in these eggs would obviously be the result of the heat alone.

A cytological study of eggs from the different sets of experiments has been made and is presented in the following pages.

II. *Material and Methods.*—The technique of the experiments is extremely simple and hardly varies at all from that used in

producing artificial parthenogenesis. A small flask of sea water is partially immersed in a beaker of water, and warmed over a flame. The eggs are put into this flask, and can easily be kept at any desired temperature for an hour or more. The temperature most frequently used was that which had been most successful in inducing parthenogenetic development, 32° to 34° C. The length of the exposure was different, however, for while the unfertilized eggs are not injured by being kept at this temperature for sixty, or even ninety minutes, the fertilized eggs appear more sensitive and should not be heated for more than thirty-five or forty minutes. In one series of experiments the eggs were placed in the warm water within five minutes after insemination; in the others they were watched until maturation was complete, and transferred to the flask before the pronuclei had united.

Eggs were preserved from the various experiments at different intervals, and a large amount of normal material was also preserved. Bouin's fluid was used as a fixative and the eggs were embedded in paraffin in the usual way. The sections were cut ten microns in thickness and stained with iron hematoxylin.

III. *Cytological Study.*—A few points in the normal and parthenogenetic development of *Cumingia* should be reviewed here for the sake of clearness. The haploid number of chromosomes is eighteen; they are very distinct in the first maturation spindle. There is no reason to suppose that the exact diploid number is not present in the first cleavage spindle of normally fertilized eggs, but owing to the form of the chromosomes, accurate counting is impossible. The long thin threads are so much intertwined, that individuals cannot be distinguished. In the parthenogenetic egg, however, the case is different. Here the chromosomes of the first cleavage are small and very definite bodies and it is possible to count them. Since the chromatin of the egg has not undergone reduction, one would expect to find thirty-six chromosomes in the first cleavage. Such is not the case, however; the number is variable, but always greater than thirty-six. Fifty to sixty of these small masses of chromatin are present, and it is evident that they do not represent individuals of the normal chromosome group. Under the experimental conditions a new distribution of the chromatic material has taken place,

and an abnormal number of chromosomes is formed. It is not possible to determine whether the mass of chromatic material rep esented in one of these plates of chromosomes is exactly equal to that of a normal cleavage plate, but on theoretical grounds it was assumed that such is the case.

(*b*) *Suppression of Maturation in Fertilized Eggs.*—As has been said the fertilized eggs in which maturation is to be suppressed are heated within five minutes after their insemination. At this time, the polar spindle has moved to the periphery of the egg, but the formation of the polar body itself has not begun. The sperm head is within the egg, but has not been transformed into a vesicular pronucleus. The first stage that is of interest in eggs preserved from these experiments is the anaphase represented in Fig. 1. In this it will be seen that a division of the eighteen bivalent chromosomes has taken place, and that the daughter chromosomes are moving somewhat irregularly to the poles of the spindle, while the sperm head is beginning to enlarge to form a vesicle.

A later stage of this nuclear division is shown in Fig. 2. Here the chromosomes have begun to form vesicles preparatory to passing into the resting stage. It will be seen from Fig. 1 that although the division of the chromosomes is complete, the migration of the daughter chromosomes to the poles of the spindle is somewhat irregular, and it is owing to this fact that we do not find two perfectly definite groups of vesicles in such a stage as Fig. 2 represents. In this figure the male pronucleus is also to be seen; it is well advanced in its development, and apparently has not been injured by the heat.

Further development of the resting nuclei is seen in Fig. 3. Here two daughter nuclei are found, with a third small vesicle which would undoubtedly have fused ultimately with them, as there is no evidence that any such vesicles are ever left out. In this stage the male pronucleus had developed still farther, and it is probable that the aster represented in the figure is of male origin.

Fig. 4 represents the final stage of the suppression of the polar body. In this, the two daughter nuclei formed from the chromosomes of the polar spindle have fused completely. The male pronucleus has reached a large size and lies closely apposed

to the egg nucleus. The aster has already divided and the spindle is forming before the male and female pronuclei have fused. In some cases, apparently, the pronuclei do not unite at all; and one finds such a prophase of the first cleavage as that represented in Fig. 5. Here two skeins of chromatin are present in the spindle, and the figure shows what it is important to note, namely, that even if the pronuclei do not unite before cleavage, all chromatic material is included in the formation of the chromosomes of the first cleavage spindle.

We can trace, then, in eggs which have been heated immediately after fertilization, a suppression of maturation which leads to the formation of two daughter nuclei within the egg. Further, we can trace the union of both these nuclei with the male pronucleus, leading to the formation of a cleavage spindle which should contain eighteen chromosomes from the sperm and thirty-six from the egg. This suppression of maturation is exactly like the process which has been seen to take place in the unfertilized eggs, in which, owing to the absence of the sperm, the result is a diploid instead of a triploid amount of chromatin.

(*c*) *The First Cleavage of Heated Eggs.*—The first cleavage spindle forms normally in the eggs which have been heated immediately after insemination. Before its formation the eggs have been returned to cooler sea water, so that conditions surrounding them are normal during the division. The important variation from the usual condition is seen in the chromatic part of the figure. Theoretically, of course, there should be fifty-four chromosomes, but as a matter of fact there are sometimes fewer, sometimes more than this number. Figs. 6 to 9 show plates of chromosomes from such eggs, in which the numbers are 44, 48, 50 and 61, respectively.

It is in a comparison of these plates with those from parthenogenetic, normal and dispermic eggs that the greatest interest is to be found. The equatorial plates of the first cleavage of normal eggs show how much the form of the chromosomes is modified by the experimental treatment. In the normal eggs (Figs. 10 and 11), one finds long threads so much twisted about each other that accurate counting of them is impossible. This is the case also in the dispermic egg, as shown in Fig. 12. It is evident that

more chromosomes are present here than in the normal egg, but the exact numerical relations cannot be determined. The most interesting comparison to be made is with the parthenogenetic egg. Here, as has been said, the chromosomes are so distinct that they can be counted, and the number is about the same as that found in fertilized eggs in which the polar bodies have been suppressed. Figs. 13 and 14 are from the first cleavage of parthenogenetic eggs, in which the numbers of the chromosomes are 46 and 47 respectively. In other cases as many as sixty-one chromosomes have been found (Morris, '17). The general shape of the bodies is similar in parthenogenetic and in heated fertilized eggs; spherical bodies and short thick rods are found instead of the normal threads. A striking difference is seen, however, in the size of the individual chromosomes, which is considerably greater in the fertilized than in the parthenogenetic egg. This is most clearly respresented in text-figure 1, in which the chromosomes are arranged in rows, graded according to size. *A* represents the chromosomes of the parthenogenetic egg, *C* those of the fertilized egg in which maturation has been suppressed. The smallest members of the two groups are about equal in size, but almost half of those in the fertilized egg are larger than the largest from the parthenogenetic one. A natural explanation of this difference in size is suggested by the fact that while the suppression of the polar body gives the parthenogenetic egg a diploid amount of chromatin, the same process makes the fertilized egg triploid. As the number of chromosomes is the same in the two cases, we might expect their size to be greater in the egg that contains the larger amount of chromatin. This was, in fact, the explanation given in a preliminary report (Morris, '17), but further experiments have shown that it is not entirely correct.

A control series of experiments was made in which eggs were subjected to heat after the formation of the polar bodies had been completed. In these, the effects of heat are shown, separated from any effect of the retention of the polar nucleus. The comparison of the parthenogenetic eggs with those that have been fertilized and heated before maturation leads one to expect that eggs heated after maturation will show cleavage chromosomes exactly like those of parthenogenetic egg, since both are

diploid and have been exposed to heat. When, however, examination of the preserved material is made, an unexpected condition is found. As in other eggs which have been heated, the chromosomes are fifty-five to sixty-five in number, but instead of being like those of the diploid parthenogenetic egg in size, they are not to be distinguished from those of the triploid egg. The reader is referred again to text-figure I in which the nuclear condition of the three kinds of eggs is shown. *A* is the parthenogenetic egg with thirty-six chromosomes incorporated into its cleavage nucleus, *B* the normal one also containing thirty-six, and *C* the triploid egg with fifty-four chromosomes. The range of size of the chromosomes is about the same in both kinds of fertilized eggs (*B* and *C*) notwithstanding the different amounts of chromatin present in them. Plates of the eggs heated after maturation are shown in Figs. 15, 16 and 17, the numbers of the chromosomes being 55, 65, and 66 respectively.

(*d*) *The Value of the Polar Body.*—The unexpected size relation of the cleavage chromosomes suggests the possibility that the chromatin of the polar nucleus is not, as a matter of fact, active. If that were the case, the parthenogenetic egg would be actually haploid, and both fertilized eggs diploid, and this would explain the condition illustrated in the text-figure. It must be remembered, however, that the polar nucleus is like the egg nucleus in appearance, and that the two vesicles fuse without any evidence of elimination of nuclear material. Dead chromatin is sometimes found in the cytoplasm of other kinds of eggs under abnormal conditions, but no such masses of chromatin are found in the material preserved from these experiments. Since this is so, it is difficult to believe that the polar nucleus, which was formed from perfectly normal daughter chromosomes, suffers complete degeneration. This is especially true when one reviews the experiments (Morris, '17) by which it was shown that the retention of the polar nucleus in unfertilized eggs is followed by normal cleavage; while those eggs from which it is extruded are unable to develop.

Study of the later stages of the development of heated eggs confirms the belief that the polar nucleus is an active element in

development. In the cleavage which occurs about three hours after fertilization, one finds that the chromatin has returned to the normal condition. The number of the chromosomes can no longer be distinguished accurately, as they are small and crowded

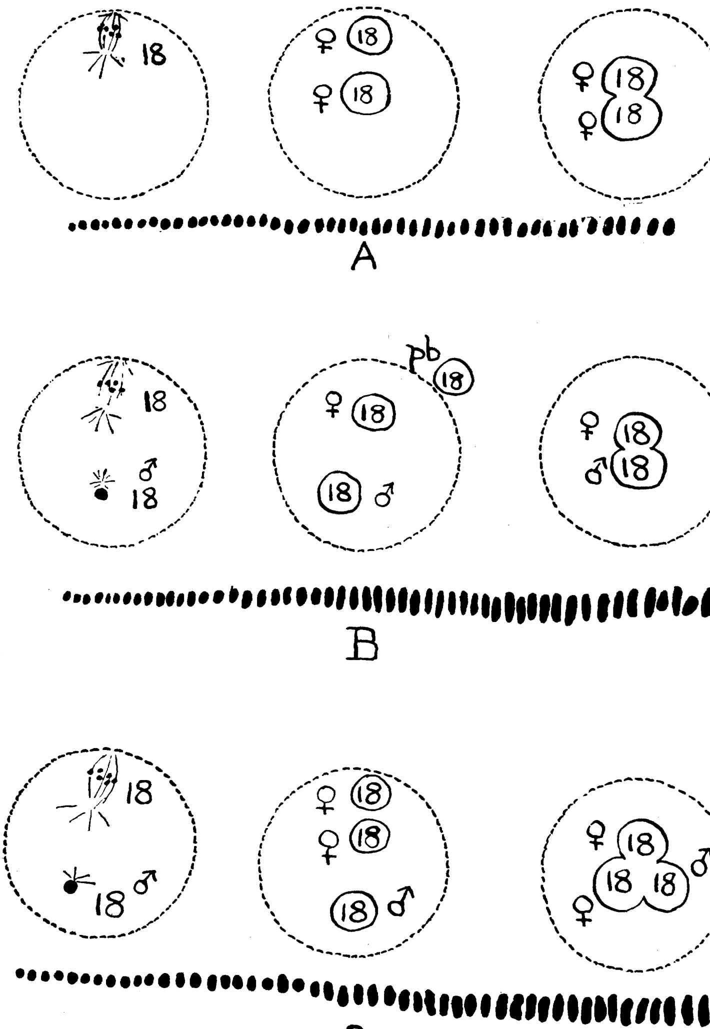

FIG. 1. Diagrams showing the nuclear conditions and the chromosomes of eggs heated at different periods. *A*, heated before fertilization (parthenogenetic); *B*, heated after fertilization and maturation; *C*, heated after fertilization and before maturation.

together in the spindle. Fig. 18 is from a fertilized egg in which maturation has been suppressed, Fig. 19 from a normal one, and Fig. 20 from one which was heated after its polar bodies had been formed. A comparison of the three figures shows that there are approximately the same number of chromosomes in the plates represented in 19 and 20, while 18 shows a larger number. This is in accordance with the belief that the polar nucleus contributes normal chromosomes to the egg.

Further evidence is given by a study of the resting nuclei. Boveri ('05) has shown that the surface area of a resting nucleus is proportional to the number of chromosomes in it, and this fact is frequently used in determining the relative amounts of chromatin when the cleavage chromosomes cannot be counted. In the present instance, the four cell stage of the various types of egg has been selected for comparison. Here we can be sure of comparing equivalent nuclei, as the size and position of the cells makes them easily recognizable. It will be remembered that the cleavage in *Cumingia* is not equal. The first division separates a small cell *AB* from a larger one, *CD*. Then follows a division of *AB* giving rise to a three-cell stage; and next *CD* divides. In the four-cell stage *A*, *B* and *C* are approximately equal, while *D* is larger than any of them. In the normal egg, shown in text-figure 2, *A*, the nuclei of all four cells are equal. Fig. 2, *B*, shows a parthenogenetic egg, with nuclei like those of the normal one; and such nuclei are also found in eggs heated after maturation, as shown in Fig. 2, *C*. All these eggs contain the diploid amount of chromatin, which is not actually changed by the heating, and, as far as can be judged from the resting nuclei, the chromatin supplied by the polar nucleus is equivalent to that supplied by the sperm. Similarity is also found among triploid eggs, whether these contain an extra sperm or a suppressed polar nucleus. Text-figure 2, *D*, shows a dispermic egg with two multinucleate cells and one nucleus abnormally large. Text-figure 2, *E*, represents a fertilized egg in which the polar nucleus was retained; here also are two multinucleate cells. In *F* which is from an egg of the same sort, the cells are not multinucleate, but two of the nuclei are above the normal size.

(*e*) *The Size and Number of the Chromosomes.*—From the

evidence given above I believe we may conclude that the parthenogenetic egg contains a diploid amount of chromatin, while the fertilized egg in which maturation has been suppressed is triploid. It is evident then that the size of the chromosomes in heated eggs is determined by some other factor than the amount

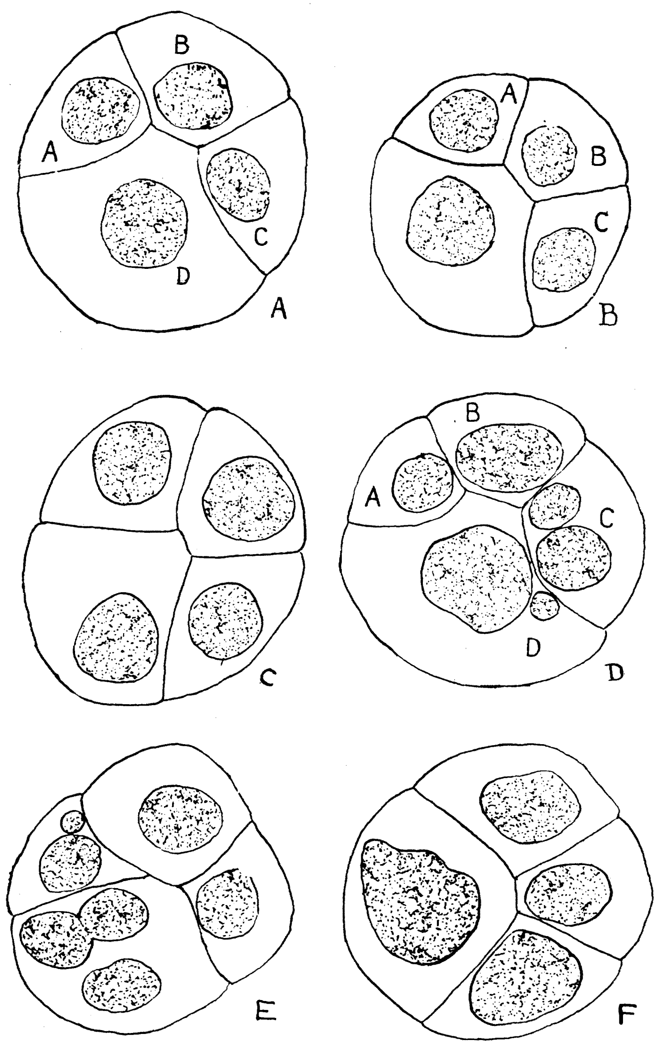

FIG. 2. *A*, normal egg (diploid); *B*, parthenogenetic egg (diploid); *C*, fertilized egg heated after maturation (diploid); *D*, dispermic egg (triploid); *E* and *F*, fertilized egg heated immediately after insemination (triploid).

of nuclear material that is present in them. If this were not so, the chromosomes of the fertilized egg heated after maturation would be like those of the parthenogenetic egg, instead of like those of the triploid one. Various other facts indicate that the appearance of these equatorial plates is not a reliable measure of the amount of chromatin present. Compare, for instance, Figs. 15 and 17. There are ten more chromosomes in the second plate than in the first, but the increase in number has evidently not been accomplished by the subdivision of some members of the first group for the average size of the bodies is greater in the plate which contains the larger number. Compare Fig. 17 with Fig. 10 also. Fertilization and maturation were normal in both these eggs, so that they undoubtedly contained the same amount of chromatin, but it seems as if there were more in the figure from the egg which has been heated. There is, in fact, no increase in amount, for in later stages the chromatin returns to the normal condition (Figs. 19 and 20).

Apparently, in heated eggs, we have an abnormal condition of aggregation of the chromatin, varying somewhat in individual cases. The variations observed in the number and size of the chromosomes are due to differing degrees of susceptibility to heat on the part of the eggs. When the susceptibility is slight, there is an increase in the number of bodies formed from the chromatin; when it is greater, there is an apparent increase in the amount of this material. It may be noted that the slighter abnormality is found in eggs heated before fertilization. At this period few eggs are destroyed by the heat, even if the exposure is prolonged to ninety minutes. After fertilization and maturation have taken place, it is difficult to warm the eggs for even thirty minutes without killing a large number; and it is in the chromosomes of these eggs that the greatest degree of abnormality is observed.

Discussion.

In 1910, Jordan studied the normal cytology of *Cumingia*, and concluded that in this animal the chromosomes are not persistent and individual cell organs. He based his conclusion on two facts; first, that they cannot be followed through the resting stage of the cell cycle; and second, that they undergo

changes of form during the development of the egg. At first sight, the evidence given in this paper seems to agree with Jordan's conclusion. If the persistence or recurrence of a definite group of chromosomes at each mitosis is essential to proving them individuals, the bodies in *Cumingia* cannot be so designated, since an entirely abnormal arrangement of the chromatin may be substituted for the usual group without injury to the egg. This use of the word "individuality" (implying unity and a separate, definite existence) has been made by many writers, and has led to much argument and confusion. It is not, however, the sense, in which all supporters of the theory of the individuality of the chromosomes have applied it. As McClung ('17) for instance, uses it, it means that each chromosome is qualitatively different from the others in the group—"a single thing of a given kind." The essential point is that the chromatin is "differentially organized and linearly arranged," and the chromosomes are aggregates of this substance, individual in the sense that they have specific characters. This theory is broad enough so that Hance's work (1918) really supports it. Hance finds, both in *Œnothera scintillans* and in the pig, certain fragmented chromosomes. The parts of chromosomes behave like normal bodies in the various stages of mitosis, and such facts would be in opposition to the narrower view of the individuality of the chromosomes. Hance, concludes "If the theory of the individuality of the chromosomes can only recognize a strict morphological continuity then the chromosomes in *Scintillans* lose their individuality through breaking up. If the theory is broader in its scope and admits an individuality not only of whole chromosomes, but of the chromatin or chromomeres, the scintillans situation falls within its limits."

The conditions found in *Cumingia* give no information with regard to the organization of the chromatin or the individuality of chromomeres. All that can be established from them is that the aggregation of the chromatin into definite bodies is subject to a certain amount of variation under normal conditions, and to very great alterations under the influence of heat. This behavior is entirely consistent, so far as I can see, with the belief that the chromatin is definitely organized and that the normal chromo-

somes are qualitatively different from each other. On the other hand the facts are not incompatible with the opposing view that the chromatin is a homogeneous substance, which condenses at mitosis into masses devoid of individuality.

Summary.

1. If the eggs of *Cumingia* are subjected to heat immediately after fertilization, they do not form polar bodies. The chromosomes of the first polar spindle divide and two resting nuclei are formed. These nuclei fuse with each other and with the male pronucleus, giving rise to a cleavage nucleus which contains a triploid amount of chromatin.

2. The equatorial plates of these eggs show forty-five to sixty chromosomes. About the same number has been found in the cleavage of parthenogenetic (diploid) eggs; but there the individual bodies are smaller than they are in the triploid eggs.

3. If fertilized eggs are heated after they have formed polar bodies, the equatorial plates of their first cleavage contain fifty-five to sixty-five chromosomes. These are equal in size to those of a triploid egg.

4. Study of later stages (resting nuclei and equatorial plates) shows that the chromatin of the polar nucleus is active, and approximately equivalent to that of a male pronucleus.

5. The size and number of the chromosomes in heated eggs is not dependent on the amount of chromatin that is present. They vary with the susceptibility of the egg to heat.

6. The conditions described are not in opposition to the theory of the individuality of the chromosomes, if that theory is given a broad interpretation.

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EXPLANATION OF PLATE I.

All the figures on this plate are from fertilized eggs, heated immediately after insemination.

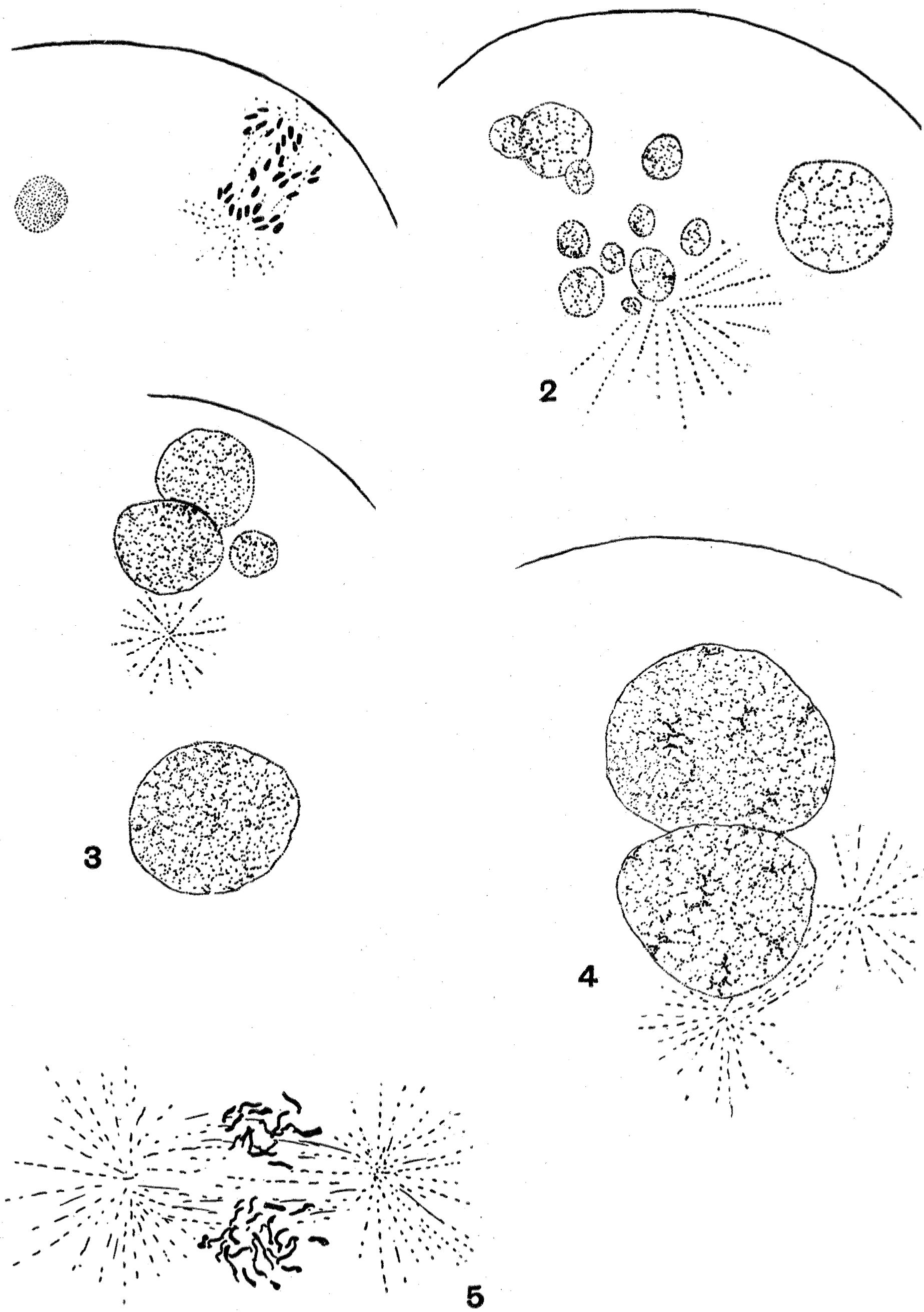
FIG. 1. First polar, spindle, anaphase, male pronucleus at the left.

FIG. 2. First polar, spindle, telophase. Male pronucleus at the right.

FIG. 3. Daughter nuclei from maturation division, male pronucleus below.

FIG. 4. Diploid female pronucleus and normal male pronucleus uniting.

FIG. 5. Prophase, first cleavage.



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EXPLANATION OF PLATE II.

FIGS. 6, 7, 8, 9. Equatorial plates of first cleavage of eggs, heated immediately after insemination (triploid).

FIGS. 10, 11. Chromosomes from first cleavage of normal eggs.

FIG. 12. Chromosomes from first cleavage of dispermic egg.

FIG. 13, 14. Equatorial plates of first cleavage of parthenogenetic eggs (diploid).

FIGS. 15, 16, 17. Equatorial plates of first cleavage of eggs heated after maturation was completed (diploid).

FIG. 18.. Chromosomes from an egg heated immediately after insemination. Egg preserved three hours after fertilization.

FIG. 19. Chromosomes from a normal egg, preserved three hours after fertilization.

FIG. 20. Chromosomes from an egg heated after maturation was completed. Egg preserved three hours after fertilization.

FIG. 21. First cleavage (anaphase) of an egg heated after maturation was completed.


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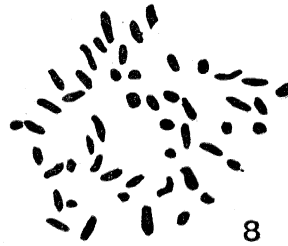
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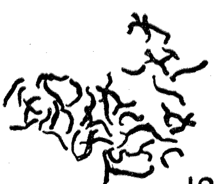
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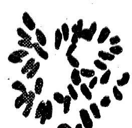
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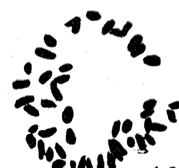
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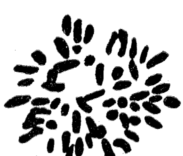
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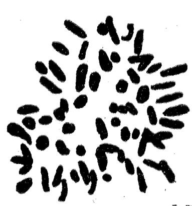
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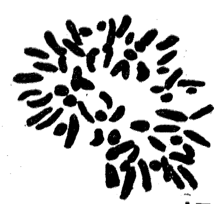
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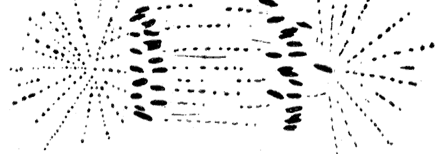
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